

**ADDITIONAL CHARACTERS FOR SEPARATING ADULTS OF
PAPILIO DEMOLEUS STHENELUS W.S. MACLEAY, 1826
(LEPIDOPTERA: PAPILIONIDAE) FROM *P. DEMOLEUS* L.
SUBSPECIES OF BIOSECURITY CONCERN TO AUSTRALIA**

JOHN E. NIELSEN

*C /- Pathway Surveillance and Operational Science, Department of Agriculture and Water
Resources, GPO Box 858, Canberra, ACT 2602*

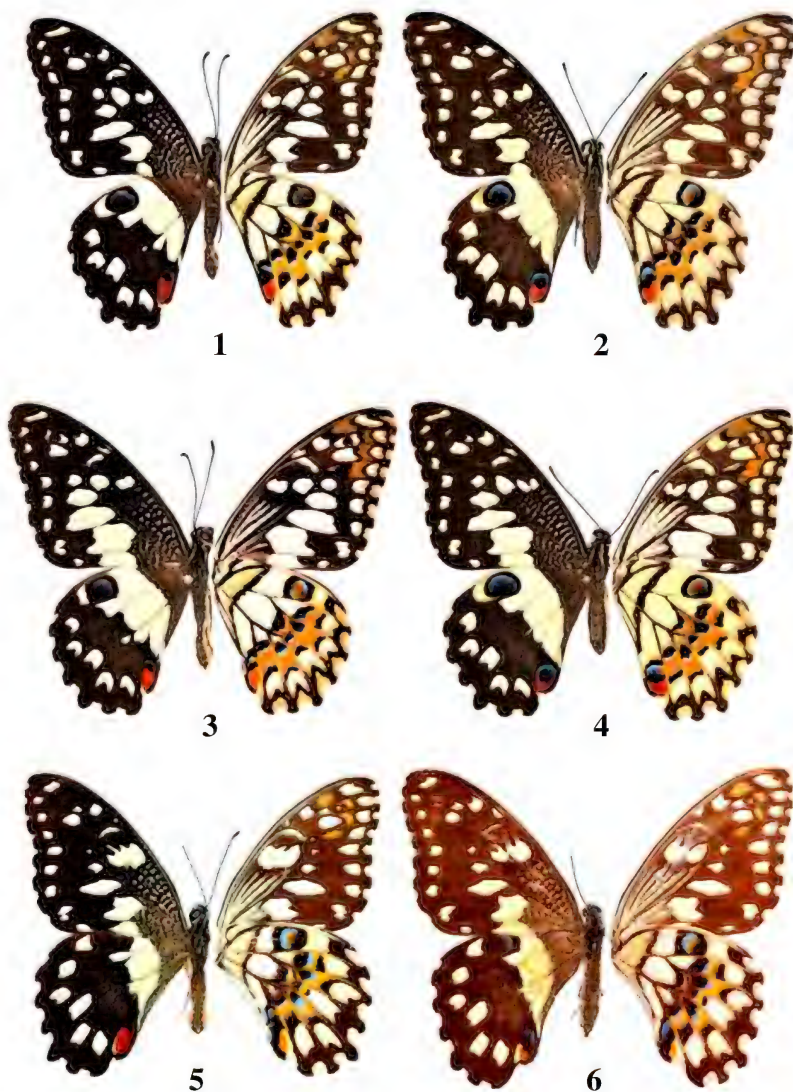
Abstract

Papilio demoleus demoleus Linnaeus and *P. d. malayanus* Wallace are exotic pests of biosecurity concern to Australia that have recently spread through the Indonesian Archipelago and New Guinea, with *P. d. malayanus* recently recorded from Australia in the Torres Strait. Although these exotic taxa are usually separable from the Australian subspecies (*P. d. sthenelus* W.S. Macleay), some specimens of the latter subspecies were found to have characters that may cause them to be misidentified as an exotic subspecies. Additional characters on the hindwing underside are demonstrated to have diagnostic utility and are presented as a contribution to the identification of *P. demoleus* in Australia for biosecurity purposes.

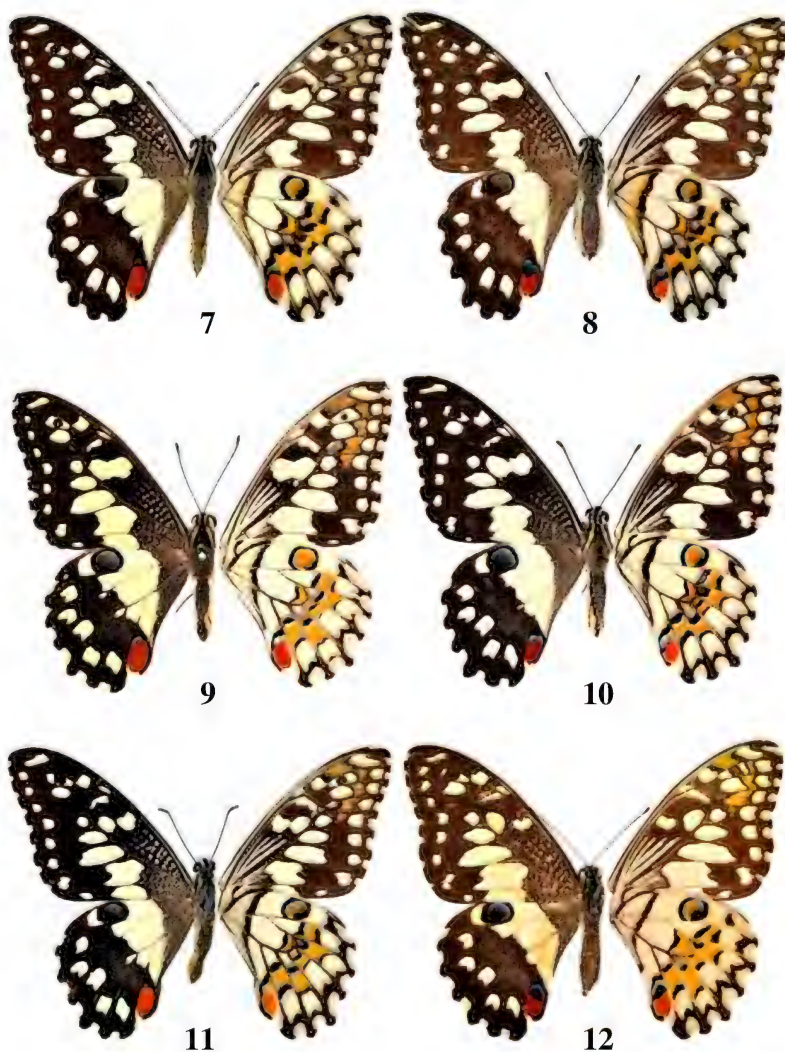
Introduction

Papilio demoleus Linnaeus, 1758 is one of the most widespread members of the Papilionidae, with five subspecies (Figs 1-12) distributed from the Middle East through subtropical and tropical Asia south to Papua New Guinea and Australia (Igarashi 1979, Smith and Vane Wright 2008, Tsukada and Nishiyama 1982). Common names of this butterfly vary with region, but include lime or citrus butterfly in Asia and chequered swallowtail in Australia (Corbet and Pendlebury 1992, Orr and Kitching 2010).

In recent years, *P. d. demoleus* (Figs 1-2) and *P. d. malayanus* Wallace, 1865 (Figs 3-4) have expanded their ranges, with *P. d. malayanus* spreading throughout the Indonesian Archipelago into Papua New Guinea (Tennent *et al.* 2011, Morgun and Wiemers 2012). *Papilio d. demoleus* has also spread through Indonesia via the Philippines and has now reached Ceram in the Moluccas (Fig. 1). These range expansions are apparently due to the clearing of rainforest for human development and associated plantings of *Citrus* in the Philippines and Sumatra, creating suitable habitat for *P. d. demoleus* and *P. d. malayanus*, respectively (Matsumoto 2002). Subsequent dispersal of both subspecies has been relatively rapid, presumably aided by the strong flight of adults and possibly by movement of nursery stock within islands. Larsen (1984) inferred that the spread of *P. d. demoleus* to the Middle East was probably also facilitated by plantings of *Citrus*. *Papilio d. malayanus* reached the Bismarck Archipelago by 2005 (Tennent *et al.* 2011), is recorded from Christmas Island (Braby 2004) and recently extended its distribution to Torres Strait, Australia (Lambkin 2017). Elsewhere, butterflies released for weddings have been implicated in the introduction of *P. d. malayanus* to the Caribbean during the early 2000s (Eastwood *et al.* 2006), while unspecified trade pathways were believed to have transported a specimen of *P. d. malayanus* to Europe (Morgun and Wiemers 2012).



Figs 1-6. *Papilio demoleus* subspecies *sensu* Smith and Vane-Wright (2008) exotic to Australia and Papua New Guinea: (1-2) *P. d. demoleus*: (1) ♂, Ambon Island, Indonesia, June 2012, local collector via H. Detani & D. Cassatt [JENC]; (2) ♀, Malalag, Mindanao Island, Philippines, ex pupa 10 May 2013, reared ex ova on *Citrus* spp, L.R. & J.P. Ring [JENC]. (3-4): *P. d. malayanus*: (3) ♂, (4) ♀, Denpasar, Bali Island, Indonesia, January 2010, H. Detani via D. Cassatt [JENC]. (5-6): *P. d. sthenelinus*: (5) ♂, (6) ♀ [both ex G.A. Waterhouse collection, AMC].



Figs 7-12. *Papilio demoleus* subspecies endemic to Papua New Guinea and Australia and an example of a mislabelled specimen: (7-8) *P. d. novoguineensis*: (7) ♂, (8) ♀, 'mrsby' [Port Moresby], ex W.W. Brandt collection [ANIC]. (9-10) *P. d. sthenelus*: (9) ♂, (10) ♀, Buderim, Queensland, 26.682070°S, 153.085875°E, 10 September 2000, J.E. Nielsen, collected from north-south migratory flight. (11) example of *P. d. sthenelus* ♂ that superficially resembles an exotic subspecies, Prison farm, Glen Innes, New South Wales, July 1969-December 1970 [ANIC]. (12) *P. d. malayanus* ♀ misidentified as *P. d. sthenelus*, bearing data 'Yeppoon, Queensland, 19 July 1962, J.C. Le Souef' and presumably mislabelled [ANIC].

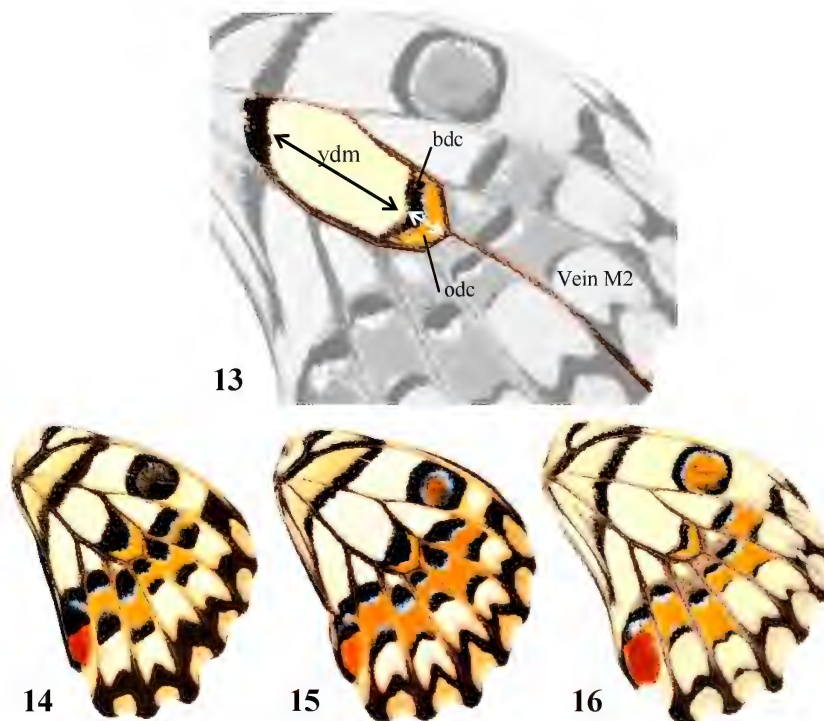
Papilio demoleus demoleus and *P. d. malayanus* are pests of biosecurity concern for Australia and are targeted through surveys performed by the Northern Australian Quarantine Strategy (NAQS; Department of Agriculture and Water Resources, unpublished). Both taxa primarily feed on *Citrus* and can be pests of economic importance (CABI 2015, Corbet and Pendlebury 1992). In contrast, *P. d. sthenelus* W.S. Macleay, 1826 (Figs 9-10) feeds on Fabaceae (*Cullen* Medik. and *Psoralea* L.), with few records from *Citrus* (including pers. obs.) and one oviposition record on *Melicope* J.R.Forst & G.Forst. (Braby 2000, Straatman 1962, Valentine *et al.* 1988). Similarly, the poorly known *P. d. novoguineensis* Rothschild, 1908 (Figs 7-8) is a Fabaceae specialist endemic to Papua New Guinea (Fenner and Lindgren 1974). The life history and biology of a fifth subspecies, *P. d. sthenelinus* Rothschild, 1895 (Figs 5-6) from the Lesser Sunda Islands, Indonesia, remains unknown (Matsumoto 2002, Lambkin, 2017).

Smith and Vane-Wright (2008) provided a key using wing pattern characters to identify *P. demoleus* subspecies. In that key, *P. d. sthenelus* is separated from *P. d. demoleus* and *P. d. malayanus* by the pale marking on the forewing discocellular space. In subspecies of *P. demoleus* that are exotic to the Australian region, this marking is divided into two spots by an area of black scaling >0.5 mm wide (hereafter ‘divided discocellular forewing spot’). In *P. d. sthenelus* this marking is considered to be not divided (Smith and Vane-Wright 2008). While examining *P. demoleus* specimens in the Australian National Insect Collection (ANIC), several specimens identified as *P. d. sthenelus* were found with a divided discocellular forewing spot. These specimens pre-dated the spread of *P. d. demoleus* and *P. d. malayanus* through Indonesia and were not linked to any import pathway. In comparing these specimens with *P. d. demoleus* and *P. d. malayanus*, it was noted that several ventral hindwing markings that were not considered by Smith and Vane-Wright (2008) might have diagnostic utility. This paper examines the utility of these characters in clarifying the identity of *P. demoleus* specimens collected in northern Australia.

Materials and method

The taxonomic arrangement used here follows Smith and Vane-Wright (2008). Specimens of all subspecies of *P. demoleus* were examined in the Australian National Insect Collection (ANIC: 1389 specimens), the Australian Museum insect collection (AMC) and the author’s private collection (JENC). Additional specimens of *P. d. demoleus* and *P. d. malayanus* were obtained from commercial sources, with the identity of these specimens confirmed using Smith and Vane-Wright (2008). The hindwing undersides of available specimens of *P. d. sthenelus* in the ANIC, and of *P. d. demoleus* and *P. d. malayanus* in the JENC, were photographed using a Nikon D90 DSLR with either a Nikkor Micro 105 mm handheld or a 40 mm Nikkor Micro lens and with the camera mounted on a copy stand and

controlled using Digicam Control software. The digital editing software GIMP was used to post-process all photos. Two subspecies, *P. d. novoguineensis* and *P. d. sthenelinus*, were not considered for analysis due to insufficient numbers of specimens being available.



Figs 13-16. *Papilio demoleus* subspecies, hind wings: (13) characters used and the measurement for morphometric analysis: bdc: black discocellular crescent; odc: ochre discocellular crescent; ydm: yellow discocellular marking; measurement of (bdc+odc): ydm is (white double headed arrow); black double headed arrow. Note that measurements are taken parallel to an axis formed by vein M2. (14-16) comparison of ventral hindwing markings of diagnostic value in separating *P. d. sthenelus* from subspecies of *P. demoleus* either endemic to Australia or of biosecurity concern: (14) *P. d. demoleus*; (15) *P. d. malayanus*; (16) *P. d. sthenelus*. Specimens are ♂♂ in JENC: see Figs 1, 3 and 9 for collection data.

Measurements were taken of two characters for each specimen using the 'measure tool' in GIMP (Figs 13-16). The first character was the combined width of the black marking at the distal apex of the discocellular cell (black discocellular crescent; bdc) and the ochre-coloured crescent adjacent to the bdc (ochre discocellular crescent; odc). The width of these markings was

measured along an axis formed by vein M2, from where M2 intersects with the discocellular cell. The second character, the length of the pale central marking of the hindwing ventral discocellular cell (yellow discocellular marking; ydm), was also measured at its widest point along the same axis. Specimens in which any marking being scored was obscured or missing due to damage or aberration were excluded from analysis. Pairwise comparisons of this ratio were made between *P. demoleus* subspecies using two-tailed Mann-Whitney U tests with a confidence interval of 0.01. It is assumed that no collector bias exists towards the characters considered here (*i.e.* the range of markings exhibited by the specimens examined are representative of *P. demoleus* populations generally). The ratio bdc:odc was also considered for the same taxa using the above method.

Available specimens of *P. d. sthenelus* in the ANIC were also surveyed to find specimens that violated the forewing character states used to separate that subspecies from *P. d. demoleus* and *P. d. malayanus* according to characters used in the key in Smith and Vane-Wright (2008).

Results

The median ratio between the markings (bdc+odc):ydc was found to differ significantly ($p < 0.00001$) between all pairwise comparisons of the three subspecies of *P. demoleus* examined (Table 1, Fig. 17). The differences were so marked it was considered unnecessary to perform a protecting multivariate analysis, especially given only three categories were compared. Across the subspecies of *P. demoleus* examined, the width of the markings bdc+odc were widest relative to marking ydc in this order: *P. d. demoleus* > *P. d. malayanus* > *P. d. sthenelus*; noting there was some overlap between *P. d. demoleus* and *P. d. malayanus*. The median ratio of markings bdc:odc was found to be significantly different between populations but had limited utility due to overlap between all taxa sampled (data not shown).

A survey of specimens in the series of *P. d. sthenelus* in the ANIC (173 specimens) found 11 specimens (6% of all specimens examined) with a divided discocellular forewing spot (Fig. 11) that could have keyed to an exotic subspecies of *P. demoleus* using the key in Smith and Vane-Wright (2008). However, one of these specimens, a female labelled as having been collected at Yeppoon by J.C. Le Seouf (Fig. 12), was identified as a female *P. d. malayanus* based on its (bdc+odc): ydm ratio.

Discussion

Biosecurity programs rely on reliable diagnostic tools being available (SPHD 2015). The variability of *P. demoleus*, including variation quantified here for the character state used by Smith and Vane-Wright (2008) to separate *P. d. sthenelus* from exotic taxa, makes reliance on a single character impractical for biosecurity diagnostic purposes. For this reason, it is desirable that diagnostic tools consider a number of characters for the sake of reliability.

Table 1. Summary of Mann-Whitney U-test statistics for pairwise comparisons of the ratio (bdc+odc):ydm measured from sampled specimens of *Papilio d. demoleus*, *P. d. malayanus* and *P. d. sthenelus*.

Taxon	Sample size	Mean	Median	Mean of Ranks	U	Z	Significance
<i>P. demoleus sthenelus</i>	133	0.245	0.251	67	2128	-8.7681	p < 0.0001
- <i>P. demoleus malayanus</i>				149.5			
<i>P. demoleus sthenelus</i>				67	3724	-10.8429	
- <i>P. demoleus demoleus</i>				161.5			
<i>P. demoleus malayanus</i>	32	0.55	0.537	21.41	1635	6.40586	
- <i>P. demoleus demoleus</i>	56	0.792	0.741	57.7			

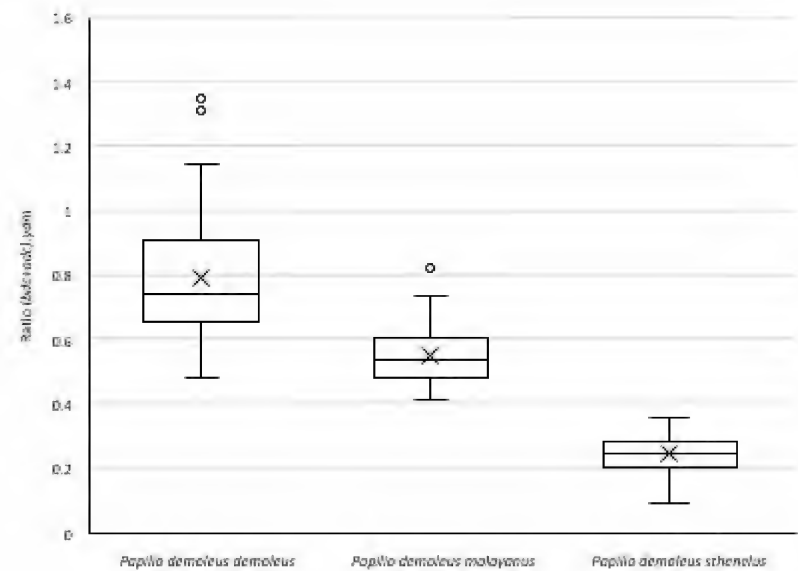


Fig. 17. Box and whiskers plot showing the spread of the ratio (bdc+odc):ydm measured from sampled specimens of *Papilio d. demoleus*, *P. d. malayanus* and *P. d. sthenelus*.

The morphometric analysis presented here demonstrates that the ratio formed by the markings (bdc+odc):ydm is reliable for separating *P. d. sthenelus* from both *P. d. demoleus* and *P. d. malayanus*, including for *P. d. sthenelus* specimens that could be confused with exotic subspecies due to variation in the forewing marking characters used by Smith and Vane-Wright (2008). It is

suggested that the (bdc+odc):ydm ratio presented here be used in addition to the characters identified in the key by Smith and Vane-Wright (2008), also illustrated by Lambkin (2017), when identifying specimens of *P. demoleus* collected in northern Australia, especially if the specimen is being used to support biosecurity decision making. In addition, molecular analysis used by several phylogenetic studies (Eastwood *et al.* 2006, Zakharov *et al.* 2004) are capable of separating the subspecies recognised by Smith and Vane-Wright (2008). Of these, Eastwood *et al.* (2006) provided sufficient resolution to be able to identify the origin of specimens introduced into the Americas. Consideration should therefore be given to using molecular tests to provide additional confidence in the identity of *P. demoleus* collected for biosecurity purposes.

The *P. d. malayanus* specimen in ANIC collected by J.C. Le Seouf bearing label data stating Yeppoon, Queensland as the collecting locality (Fig. 12) is considered to have been accidentally mislabelled and is not taken to represent early evidence of *P. d. malayanus* in Australia. Le Seouf had specimens of exotic species in his collection, including some Malaysian taxa (M.F. Braby pers. comm.) and there is evidence that other material he collected was also incorrectly labelled (Dunn 1985).

Invasive species, including numerous arthropod taxa, pose a serious threat to Australia. All entomologists, including amateur collectors, are encouraged to be aware of the contribution they can make to Australian biosecurity by collecting and reporting specimens of suspected exotic taxa. Plant Health Australia (2016) provides guidance on how to report suspect exotic plant pests if they are detected in Australian States or Territories.

Acknowledgements

Jacque Recsei (Australian Museum, Sydney) and Ted Edwards and You-Ning Su (Australian National Insect Collection, Canberra) are thanked for granting access to specimens in their care. Les and Janice Ring generously bred a series of *P. d. demoleus* for this work, while Fabian Douglas contributed additional specimens of *P. d. demoleus* and, together with Michael Braby, contributed helpful discussion regarding J.C. Le Seouf's collection. Further material of *P. demoleus* was obtained from David Cassatt, Ronald Hart and Pawel Lewenthal. Ross and Lilac Kendall provided opportunities to observe larvae of *P. d. sthenelus* using *Citrus* spp in Australia. Stacey Anderson and Luke Halling (NAQS) and Trevor Lambkin assisted with helpful discussions, while Michael Braby, Julietta Brambilla, Rod Eastwood, Ted Fenner, Albert Orr and John Tennent provided literature. Caroline Martin and Gertraud Norton assisted with departmental clearance of the manuscript, while Albert Orr generously reviewed drafts of the manuscript and, with Ted Edwards, provided suggestions with statistical analysis. I am especially grateful to my wife Haliz for supporting my work on the Lepidoptera.

References

- BRABY, M.F. 2000. *Butterflies of Australia: their identification, biology and distribution*. CSIRO publishing: Collingwood; xxvii + 976 pp.
- BRABY, M.F. 2004. *The complete field guide to butterflies of Australia*. CSIRO: Collingwood; 339 pp.
- CABI. 2015. *Crop Protection Compendium*. Wallingford, UK: CAB International. Available online (accessed 12 Jan 2015): www.cabi.org/cpc
- CORBET, A.S. and PENDLEBURY, H.M. 1992. *The butterflies of the Malay Peninsula*. Fourth edition. (Edited by J.N. Eliot). Malaysian Nature Society; x + 595 pp.
- DUNN, K.L. 1985. Specimens of interest in the J. C. Le Seouf collection of Australian butterflies. *Victorian Naturalist* **101**: 94-97.
- EASTWOOD, R.G., BOYCE, S.L. and FARRELL, B.D. 2006. The provenance of Old World lime swallowtail butterflies, *Papilio demoleus* (Lepidoptera: Papilionidae), recently discovered in the New World. *Annals of the Entomological Society of America* **99**: 164-168.
- FENNER, T.L. and LINDGREN, E. 1974. The life history and larval foodplants of *Papilio demoleus* L. (Lepidoptera: Papilionidae) in southern New Guinea. *Papua New Guinea Science Society Proceedings* **25**: 63-71.
- IGARASHI, S. 1979. *Papilionidae and their early stages*. Kondansha, Tokyo.
- LAMBKIN, T.A. 2017. *Papilio demoleus malayanus* Wallace, 1865 (Lepidoptera: Papilionidae) on Dauan Island, Torres Strait, Queensland and recent confirmation of *P. d. sthenelinus* Rothschild, 1895 in the Lesser Sunda Islands. *Australian Entomologist* **44**(2): 65-74.
- LARSEN, T.B. 1984. *Butterflies of Saudi Arabia and its neighbours*. Stacey International, London; 160 pp.
- MATSUMOTO, K. 2002. *Papilio demoleus* on Borneo and Bali. *Journal of the Lepidopterists' Society* **56**(2): 108-111.
- MORGUN, D.V. and WIEMERS, M. (2012). First record of the lime swallowtail *Papilio demoleus* Linnaeus, 1758 (Lepidoptera, Papilionidae) in Europe. *Journal of Research on the Lepidoptera* **45**: 85-89.
- ORR, A.G. and KITCHING, R. 2010. *The butterflies of Australia*. Allen & Unwin: Sydney; 336 pp.
- PLANT HEALTH AUSTRALIA. 2016. Reporting suspect pests (1800 084 881). Available online (accessed 12 Aug 2016): <http://www.planthealthaustralia.com.au/biosecurity/emergency-plant-pests/reporting-suspect-pests/>
- SMITH, C.R. and VANE-WRIGHT, R.I. 2008. Classification, nomenclature and identification of lime swallowtail butterflies: a post-cladistic analysis (Lepidoptera: Papilionidae). *Systematics and Biodiversity* **6**: 175-203.
- SPHD 2015. Operating guidelines for the subcommittee on plant health diagnostics and its working groups. Available online (accessed 12 Aug 2016): <http://plantbiosecuritydiagnostics.net.au/wordpress/wp-content/uploads/2015/06/SPHD-Operating-guidelines-June-2015.pdf>
- STRAATMAN, R. 1962. Notes on certain Lepidoptera ovipositing on plants which are toxic to their larvae. *Journal of the Lepidopterists' Society* **16**: 99-103.
- TENNENT, W.J., DEWHURST, C.F. and MÜLLER, C.J. 2011. On the recent spread of *Papilio demoleus* Linnaeus, 1758, in eastern Papua New Guinea (Lepidoptera, Papilionidae). *Butterflies (Teinopalpus)* **58**: 30-33.

TSUKADA, E. and NISHIYAMA, Y. 1982. *Butterflies of the Southeast Asian islands. Volume I. Papilionidae*. Plapac, Tokyo; 460 pp.

VALENTINE, P., FIRTH, C. and FIRTH, D. 1988. *Australian tropical butterflies*. Firth & Firth, Malanda; 71 pp.

ZAKHAROV, E.V., SMITH, C.R., LEES, D.C., CAMERON, A., VANE-WRIGHT, R.I. and SPERLING, F.A.H. 2004. Independent gene phylogenies and morphology demonstrate a Malagasy origin for a wide-ranging group of swallowtail butterflies. *Evolution* **58**(12): 2763-2782.